

## N.M.R. Spectra of Porphyrins. Part 35.<sup>1</sup> An Examination of the Proposed Models of the Chlorophyll *a* Dimer

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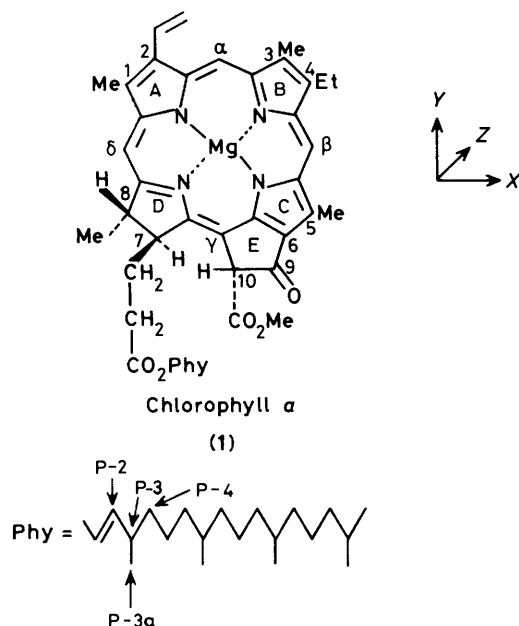
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The n.m.r. complexation shifts at 500 MHz of the protons of chlorophyll *a* have been obtained from titration experiments with [<sup>2</sup>H<sub>4</sub>]methanol and chlorophyll *a* dissolved in CDCl<sub>3</sub> at such concentrations (3mM) that only the chlorophyll dimer is present. This more accurate and more complete set of complexation shifts than obtained previously has been used to examine critically proposed models of the chlorophyll *a* dimer, using the double-dipole model of the chlorophyll ring current to calculate the dimer shifts. Proposals based on the crystal structure of ethyl chlorophyllide-*a* dihydrate (the Strouse model), on exciton-theoretical and infrared data (the Shipman model), and the various skew structures, do not lead to any agreement with the observed complexation shifts. The face-to-face proposal (the Fong model) gives the correct overall pattern for the complexation shifts, but not quantitative agreement. The unsymmetrical head-to-tail ('piggy-back') structure does give quantitative agreement with the observed shifts for all the protons measured. These last two models appear to involve co-ordination of the C-10 methoxycarbonyl C=O with the adjoining magnesium atom, which is not consistent with observations on the pyrochlorophyllide series, in which similar complexation shifts are observed. A new structure which both quantitatively fits the observed complexation shifts and which appears to satisfy these other observations is proposed. This is a back-to-back structure in which *both* the C-10 methoxycarbonyl groups in the dimer are *exo* to the dimer structure. The arrangement is not symmetric and the two chlorophyll molecules are in very different environments. In one molecule the C-9 C=O is near to the neighbouring magnesium atom and could be co-ordinated to the magnesium *via* a bridging water molecule. In the other molecule the C-7 propionic C=O is situated near to the neighbouring magnesium atom and could also be co-ordinated to it. The C-10 methoxycarbonyl groups are not involved in the dimer binding interaction.

Chlorophylls function *in vivo* both as antennae and phototrapers that harvest light and begin the process of photosynthesis in plants and photosynthetic bacteria.<sup>2</sup> The organization of the chlorophylls *in vivo* in both the antenna and photoreaction centre is crucial to our understanding of the mechanism of photosynthesis. For this reason, the aggregation of chlorophyll *a* (1) *in vitro* has been extensively investigated using a variety of

spectroscopic methods, including infrared,<sup>3</sup> visible,<sup>4</sup> resonance Raman,<sup>5</sup> fluorescence,<sup>6</sup> circular dichroism,<sup>7</sup> magnetic resonance,<sup>3,8</sup> and X-ray techniques.<sup>9</sup> These studies have, in turn, produced a number of possible models of chlorophyll aggregation and of the photoreaction centre,† most of which involve either direct or hydrogen-bonded nucleophilic interactions between the central magnesium ion and the 9-keto and/or the C-7 and C-10 methoxycarbonyl groups of a neighbouring molecule. In the crystal structure of ethyl chlorophyllide *a* dihydrate<sup>9a</sup> the chlorophyll molecules form cross linked one-dimensional polymers in which one water molecule is co-ordinated to the magnesium and the C-9 C=O of a neighbouring molecule, and the other water is hydrogen bonded to the first water molecule and to the C-7 C=O of a third chlorophyll. Infrared<sup>3</sup> and molecular weight studies<sup>11</sup> of chlorophyll in polar and non-polar solvents demonstrated the presence of aggregates in non-polar solvents which were dissociated by adding alcohol or by polar solvents, and the C-9 C=O group of chlorophyll *a* was involved in this aggregation. In contrast to the solid state however, in which the magnesium-free compounds form very similar lattice structures, in solution the aggregation is determined by the presence or absence of magnesium. Circular dichroism spectra of several chlorophylls in solution were interpreted on the basis of a skew dimer in non-polar solvents in which, unlike the crystal structure, the chlorophyll molecules did not lie in parallel planes.<sup>12</sup> In this structure the C-9 C=O oxygen of one molecule is directly bonded to the magnesium



† Contrary to early indications from e.s.r., however, the P700 photoreaction centre in plant photosynthesis is no longer considered to be a dimer.<sup>10</sup>

of the second molecule, and the C-7 propionate C=O oxygen of the second molecule interacts with the magnesium of the first.

The n.m.r. studies of Katz and co-workers<sup>3</sup> clearly identified the region of overlap of the chlorophyll *a* molecules as ring C and the exocyclic ring E. They further postulated a skew aggregate structure with the C-9 C=O oxygen bonding to the magnesium. However, the role of any adventitious water molecules in these studies was raised by Fong,<sup>13</sup> who also reinterpreted the n.m.r. data on the basis of a C<sub>2</sub> symmetric dimer in which bonding occurs *via* the C-10 C=O oxygen and the magnesium. This interpretation fails to explain the very similar aggregation behaviour of pyrochlorophyll *a*, in which the C-10 methoxycarbonyl group is missing.

A more considered special-pair symmetric dimer structure is that of Shipman *et al.*,<sup>14</sup> which involves hydrogen bonding between the C-9 C=O oxygen of one molecule and the hydroxy group of an alcohol bonded to the magnesium. This structure differs from the crystal studies in that the co-ordinating ligand is on the opposite face of the molecule to the C-10 methoxycarbonyl group. More recently, Kooyman and Schaafsma,<sup>15</sup> using n.m.r. relaxation and ring-current shifts, have suggested a perpendicular orientation of the chlorophyll molecules in the dimer in which the C-9 C=O is directly bonded to the magnesium. They, however, also used relatively concentrated solutions (0.01–0.8M), and did not consider other proposed structures.

N.m.r. spectroscopy can, in principle, distinguish between these possible structures insofar as they apply to the chlorophyll *a* aggregation in solution, and to this end we have described a model of the chlorophyll ring current<sup>16</sup> and utilized it to analyse the observed n.m.r. aggregation shifts.<sup>8</sup> A novel 'piggy back' structure for the chlorophyll aggregates was proposed in these studies but the definition of the structure and differentiation between possible models were hampered by the limitations of the existing n.m.r. data. The data were obtained on low-field spectrometers<sup>3,17</sup> and necessarily involved the use of relatively concentrated solutions (*ca.* 0.1M in CDCl<sub>3</sub>) in which the aggregation number of the aggregate was very likely greater than two. The calculation of the observed aggregation shifts and indeed even the definition of the geometry of the aggregate becomes prohibitively complex in the general case for aggregates larger than dimers (see ref. 8 for a fuller discussion). From vapour phase osmometry<sup>11</sup> the aggregation number of

chlorophyll *a* was found to be 2 in solutions <0.01M, increasing to *ca.* 4 in 0.1M solutions.

We have therefore remeasured the aggregation n.m.r. shifts of chlorophyll *a* at concentrations <0.01M in CDCl<sub>3</sub> at the highest applied magnetic field available to us (500 MHz). This increased dispersion allowed the assignment of many more of the chlorophyll protons<sup>18</sup> and thus a more complete aggregation map than previously. These new data provide a rigorous examination of previously proposed models of the aggregate structure and this is presented here.

### Experimental

Chlorophyll *a* was obtained from Sigma and dissolved in CDCl<sub>3</sub> filtered through activated alumina to remove traces of acid and water. The spectrum was obtained on a Nicolet NT-500 (500 MHz; <sup>1</sup>H) spectrometer operating at 23 °C. Typical conditions were 32 K data points, sweep width 6 kHz, giving a digital accuracy of 0.4 Hz (<0.001 p.p.m.) per point. The pulse width (90°) was 7 μs, acquisition time 2 s, and *ca.* 80 accumulations were obtained.

### Results

To avoid the presence of aggregates larger than the dimer (see the Introduction), the chlorophyll *a* spectrum was obtained at 2.8mm concentrations. The solution was then titrated with aliquots of a [<sup>2</sup>H<sub>4</sub>]methanol solution in CDCl<sub>3</sub> from 0.8 mol equiv. of [<sup>2</sup>H<sub>4</sub>]methanol to 5 μl of pure [<sup>2</sup>H<sub>4</sub>]methanol, *i.e.* a very large excess. The titration measurements are given in Table 1 and Figure 2, and some of the spectra obtained are shown in Figure 1. With a large excess of [<sup>2</sup>H<sub>4</sub>]methanol the chlorophyll spectrum (Figure 1D) is that characteristic of the monomeric species, and the well resolved spectrum at 500 MHz has been reported and completely assigned,<sup>18</sup> except for the CH and CH<sub>2</sub> protons of the phytol side chain (peaks P-5 to P-15), which resonate at 1.0–1.2 p.p.m.

However, the spectrum of chlorophyll in non-complexing solvents shows considerable broadening of many of the resonances. In 0.04M solution in [<sup>2</sup>H<sub>8</sub>]octane, virtually all traces of fine structure are absent,<sup>19</sup> whereas in CDCl<sub>3</sub> certain resonances broaden considerably; thus, even at 500 MHz, the observed spectrum (Figure 1A) is not easily assigned. The cause of this line broadening is not clear. The obvious explanation

Table 1. Titration of chlorophyll *a* (1) in CDCl<sub>3</sub> with CD<sub>3</sub>OD<sup>a</sup>

Resonance	mol equiv. of CD <sub>3</sub> OD added															+ 5 μl CD <sub>3</sub> OD
	0	0.8	1.3	2.5	4.0	4.8	6.4	8.0	9.6	12.8	16.0	20.0	28.0	40.0	56.0	
β	9.44	9.44	9.46	9.45	9.47	9.47	9.48	9.49	9.50	9.52	9.53	9.53	9.54	9.54	9.55	9.54
<i>meso</i> α	9.23	9.23	9.21	9.23	9.24	9.24	9.25	9.26	9.26	9.27	9.28	9.28	9.29	9.29	9.29	9.29
δ	8.23	8.23	8.23	8.24	8.25	8.26	8.26	8.27	8.28	8.29	8.30	8.30	8.31	8.31	8.31	8.31
10-H	4.98 <sup>c</sup>				5.40	obscured	5.60	5.68	5.77	5.91	5.98	6.06	6.14	6.19	6.21	6.24
P-2	4.77 <sup>c</sup>	4.80	4.80	4.82	4.89	4.90	4.90	4.91	4.93	4.96	4.98	5.00	5.02	5.03	5.04	5.07
8-H	3.96 <sup>c</sup>	4.00	4.02	4.06	4.12	4.14	4.20	4.23	4.26	4.30	4.33	4.36	4.37	4.38	4.39	4.39
10-OMe	3.34 <sup>b</sup>	3.40	3.41	3.47	3.55	3.58	3.63	3.68	3.72	3.78	3.82	3.86	3.89	3.92	3.94	3.98
4a-CH <sub>2</sub>	3.74	3.74	3.74	3.74	3.74	3.74	3.74	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
5-Me	3.00 <sup>c</sup>							3.39	3.41	3.48	3.52	3.56	3.60	3.62	3.63	3.64
1-Me	3.31	3.32	3.32	3.32	3.32	3.32	3.32	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.30
3-Me	3.24	3.24	3.24	3.25	3.25	3.25	3.26	3.26	3.26	3.27	3.27	3.27	3.27	3.27	3.27	3.27
P-4-CH <sub>2</sub>	1.89	1.89	1.88	1.89	1.88	1.88	1.88	1.88	1.88	1.88	1.87	1.87	1.87	1.87	1.87	1.86
8-Me	1.39 <sup>b</sup>	1.43	1.45	1.49	1.53	1.55	1.58	1.61	1.64	1.67	1.69	1.72	1.73	1.75	1.76	1.78
4b-Me	1.73	1.73	1.73	1.73	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.71	1.71
P-3a-Me	1.38	1.43	1.45	1.45	1.45	1.45	1.45	1.45	1.46	1.46	1.47	1.47	1.48			1.52

<sup>a</sup> Initial concentration 2.8mm in CDCl<sub>3</sub>. Chemical shifts relative to CHCl<sub>3</sub> at 7.260 p.p.m.; <sup>b</sup> from Figure 2; <sup>c</sup> from linear plots *vs.* 10-OMe (see text).

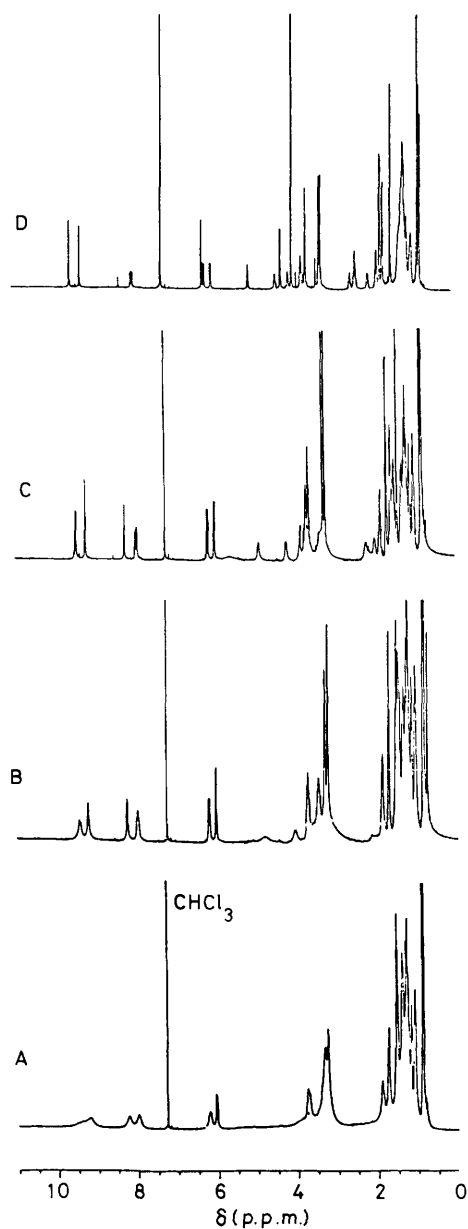


Figure 1. Titration of chlorophyll *a* (1) in  $\text{CDCl}_3$  with  $\text{CD}_3\text{OD}$  at 500 MHz; A,  $\text{CDCl}_3$  only; B, with 2.5 equiv.  $\text{CD}_3\text{OD}$ ; C, with 8 equiv.  $\text{CD}_3\text{OD}$ ; D, with 5  $\mu\text{l}$  pure  $\text{CD}_3\text{OD}$  added

that it is due to exchange broadening between different sites on the n.m.r. scale, *i.e.* monomer-dimer, *etc.*, is almost certainly correct in concentrated solutions and hydrocarbon solvents, in which large aggregates are formed, and there is no doubt that exchange broadening is occurring for some resonances during the titrations. However, this explanation cannot account for the considerable line broadening observed for the *meso* protons (Figure 1A), as these protons show very small complexation shifts ( $<0.1$  p.p.m., see later), which could not produce the line broadening observed. Waterton and Sanders<sup>20</sup> have demonstrated the very considerable line broadening in the chlorophyll *b* proton n.m.r. spectrum which occurs when small quantities of the chlorophyll  $\pi$ -cation radical are present. This broadening is, of course, greatest for the *meso* protons because the largest unpaired spin density is on the adjacent carbon atoms. However, degassing the chlorophyll *a* solutions did not affect the

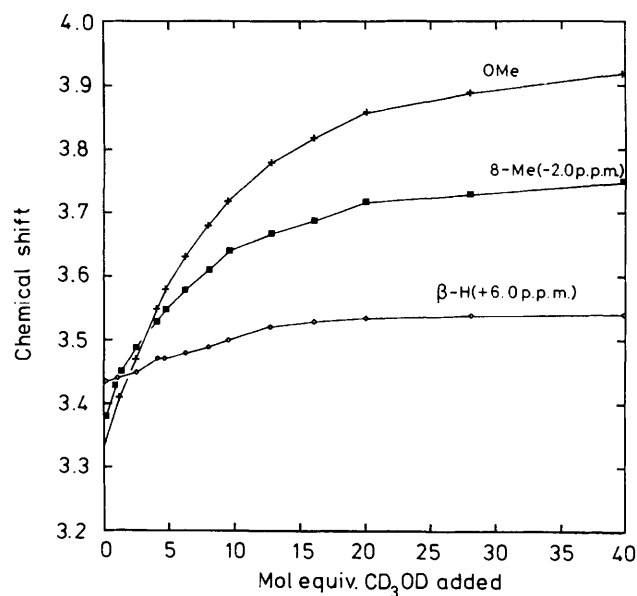
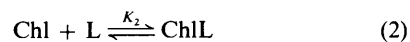


Figure 2. Titration of chlorophyll *a* (1) in  $\text{CDCl}_3$  with  $\text{CD}_3\text{OD}$ . Chemical shifts *vs.* mol equiv.  $\text{CD}_3\text{OD}$  added

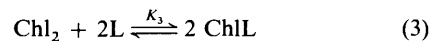
line broadening, which does decrease as soon as the [ $^2\text{H}_4$ ]-methanol is added. A further possibility is chemical shift anisotropy, which again would be most pronounced at the *meso* positions owing to their highly anisotropic environment; in some support of this mechanism, the line broadening appears greater at 500 than at 100 MHz. However, calculations based on a chlorophyll dimer with reasonable values for the anisotropy give much less than the observed line broadening.<sup>21</sup>

It is very likely that a number of factors contribute to the line broadening, which, whatever the cause, precludes the chlorophyll spectrum in pure  $\text{CDCl}_3$  from being used directly to obtain accurate values of the complexation shifts. These can, nevertheless, be obtained in a straightforward manner as follows.

We consider for simplicity only two competing equilibria, the dissociation of the chlorophyll *a* dimer, and the subsequent complexation with ligand (methanol) according to equations (1) and (2):



to give the overall equilibrium as



where  $K_3 = K_1K_2$ .

Ballschmiter *et al.*<sup>11</sup> showed by vapour-phase osmometry that chlorophyll *a* exists as a dimer in  $\text{CCl}_4$  down to infinite dilution, with higher aggregates appearing at concentrations  $>10^{-2}\text{M}$ . They also observed that dichloroethane was a more aggregating solvent than  $\text{CCl}_4$ . Thus we may safely assume that the chlorophyll *a* solution at 2.8 mM in  $\text{CDCl}_3$  contains predominantly dimers, and in support of this assumption the spectrum was unchanged at slightly higher concentration (4.1 mM).

The titration curves obtained by plotting the observed chemical shifts against concentration will be determined by the value of  $K_3$ . This value for the analogous titration of chlorophyll *a* with tetrahydrofuran in  $\text{CCl}_4$  solution was found<sup>22</sup> to be  $19.4 \text{ l/m}^{-1}$ , and this value implies that the titration curves may be

**Table 2.** Observed and calculated complexation shifts ( $\Delta\delta$ ) for chlorophyll *a* (1)

Proton	Observed shifts (monomer-dimer)			Calculated shifts					
	This work	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>
$\alpha$	0.06	0.08	0.24	0.13	0.10	0.02	0.02	0.00	0.06
<i>meso</i> $\beta$	0.10	0.18	0.22	0.06	0.31	0.24	0.36	0.22	0.07
$\delta$	0.08	0.13	0.07	0.06	0.09	0.03	0.12	0.16	0.03
1-Me	0.01	0.03	0.05	0.33	0.06	0.03	0.05	0.00	0.07
3-Me	0.03	0.07	0.11	0.08	0.11	0.02	0.05	0.02	0.06
5-Me	0.64	0.83	0.90	0.47	3.04	0.58	0.35	0.43	0.59
8-Me	0.39	0.42		0.12	0.02	0.02	0.49	0.31	0.29
8-H	0.43			0.10	0.12	0.01	0.44	0.37	0.42
10-H	1.26	1.85	2.05	0.06	0.31	1.56	1.27	1.34	1.32
4a-CH <sub>2</sub>	0.01	0.08		0.09	0.05	0.11	0.03	0.01	0.07
4b-Me	0.02			0.13	0.45	0.10	0.06	0.01	0.01
10-OMe	0.64	0.72	0.61	0.05	0.04	1.28	0.75	0.60	0.50
P-2	0.30								
P-4-CH <sub>2</sub>	0.03								
P-3a-Me	0.14								

<sup>a</sup> Methyl chlorophyllide (0.08M in CDCl<sub>3</sub>) titrated with methanol (ref. 17). <sup>b</sup> Chlorophyll *a* (0.06M in CCl<sub>4</sub>) titrated with [<sup>2</sup>H<sub>4</sub>]pyridine (ref. 3). <sup>c</sup> The Strouse model, displacement co-ordinates -5.0, 6.8, -4.0 Å, no rotation. <sup>d</sup> The Shipman model, displacement co-ordinates -5.0, -6.0, -3.6 Å, rotated -105°. <sup>e</sup> The skew dimer, displacement co-ordinates 2.6, -8.4, 0.0 Å, orthogonal position. <sup>f</sup> The Fong model, displacement co-ordinates 0.0, 3.6, 5.0 Å, C<sub>2</sub> symmetry. <sup>g</sup> the piggy back model, displacement co-ordinates 0.0, -4.5, 6.0 Å, rotated 205°. <sup>h</sup> The back-to-back model, displacement co-ordinates 3.4, -5.8, 4.8 Å, inverted molecule.

easily extrapolated to give the complexation shifts in the dimer. This is indeed the case, and Figure 2 shows the titration curves for the C-10 methoxy group, 8-H, and  $\beta$ -*meso*-H in which the resonances may be clearly assigned down to very low additions of methanol. As all the proton signals in the spectra experience similar titration curves, the only difference being the chemical shift values at the two extremes, there will be an accurately linear relationship between the chemical shift values of any two given signals. Thus, for those resonances for which it is not possible to give a definitive assignment at low concentrations of methanol, owing to either broadening or the disappearance of the signal under larger groups of resonances, we make use of this linear relationship, using the C-10 methoxy group as the base resonance to obtain the complexation shifts. This method gave accurate complexation shifts for the important 10-H and the 5-Me, both of which could not be identified at low concentrations of added methanol (Table 1). The values of the complex shifts obtained by all these methods are given in the first column of Table 1, with the method used to obtain them. The only resonances not assigned and measured in the complex, apart from the vinyl protons which, like the *meso* protons show virtually no complexation shifts, are the 7-H and P-1 CH<sub>2</sub> resonances and the 7a- and 7b-methylene resonances of the propionate side chain. The 7-H and P-1 CH<sub>2</sub> resonances overlap continuously (apart from the final spectrum, Figure 1D), and they also are obscured by the signals from the other side chain protons, thus no accurate estimate of their complexation shifts could be made. The 7a- and 7b-methylene protons form a complex four-spin system in the monomer<sup>18</sup> and no attempt was made to identify these in the titration experiments.

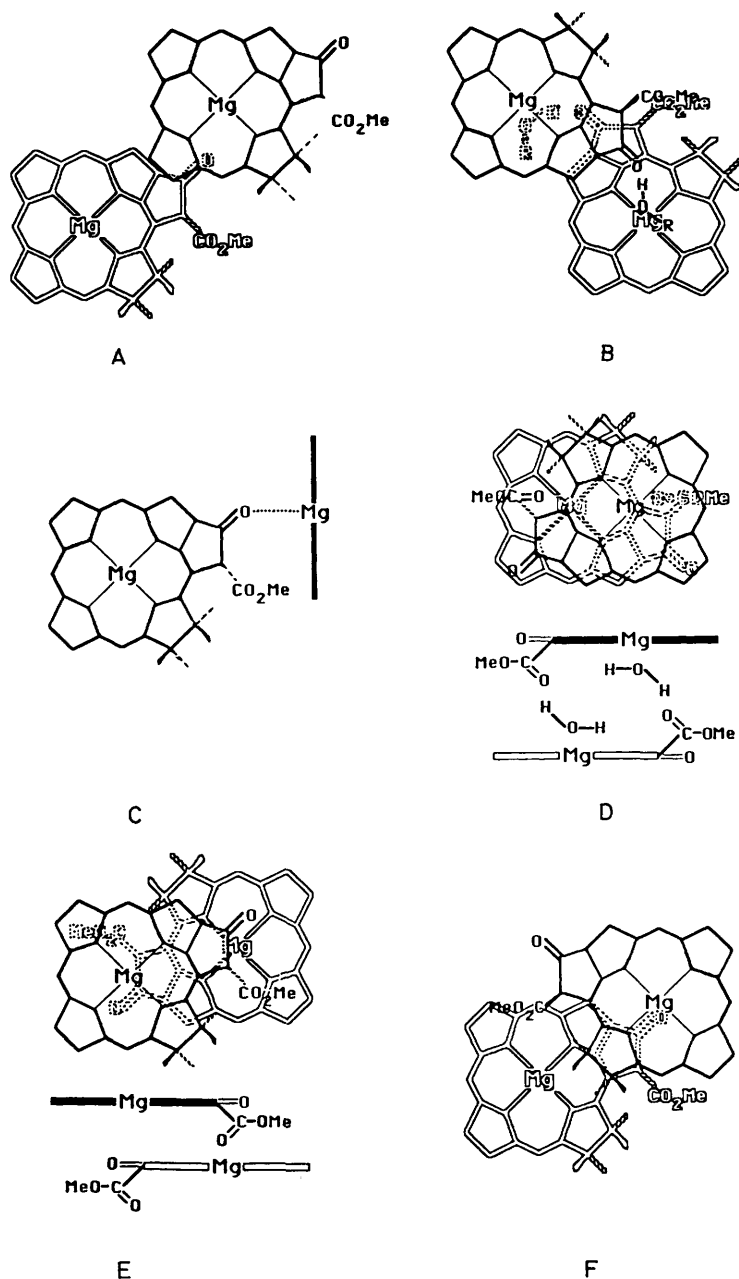
Table 1 and Figure 2 demonstrate that, as would be expected, a considerable excess of [<sup>2</sup>H<sub>4</sub>]MeOH is required to dissociate the chlorophyll complex completely. This is most clearly noticed for the 10-H resonance, which is still exchange broadened and moving with the addition of 40 mol equiv. of methanol. However, there are both specific and non-specific solvent effects on the proton chemical shifts of chlorophyll *a*,<sup>18</sup> the former possibly resulting from the different complexation states of the magnesium,\* and the latter from reaction field effects of the polar groups, *etc.* Thus, we take as the appropriate

'monomer' shifts the observed chemical shifts in CDCl<sub>3</sub> with the addition of 5  $\mu$ l of pure [<sup>2</sup>H<sub>4</sub>]methanol (Table 1).

**Models of the Dimer Geometry.**—The analysis of the chlorophyll titration experiments presented leads to more precise values of the chlorophyll dimer aggregation shifts than previously obtained. Before considering the fit of these aggregation shifts with those predicted on the basis of the various models proposed for the chlorophyll dimer, it is of interest to compare the results obtained here with those of previous workers, and this is given in Table 2. It can be seen that there is generally good agreement with the original values of Closs *et al.*<sup>17</sup> in much more concentrated solutions. The differences are undoubtedly due to the presence of higher aggregates in these more concentrated solutions, and this is clearly seen for those protons with the largest complexation shift, in particular 10-H, 5-Me, and 8-Me. Note that the limiting value of the complexation shift for a large aggregate is slightly more than twice the complexation shift in the dimer.<sup>8</sup> The large shifts observed for 10-H, particularly for the measurements in CCl<sub>4</sub> solution,<sup>3</sup> are almost twice the present values, strongly suggesting large aggregates in this less polar solvent. Having obtained what may be regarded as the correct dimer complexation shifts we can now consider the various proposed models of the chlorophyll dimer, using the ring current model previously described<sup>8,16</sup> to calculate the complexation shifts. It should perhaps be emphasized here that some of the structures were proposed as models for the photosynthetic 'special-pair',<sup>10</sup> rather than for *in vitro* dimerization which is being examined here.

**The Strouse Model.**—The crystal structure of ethyl chlorophyllide *a* dihydrate obtained by Strouse and co-workers<sup>9a</sup> consists of a series of one-dimensional polymers of chlorophyll molecules linked by a hydrogen-bonded chain involving both the C-7 and C-10 ester C=O groups and also the C-9 keto group. These one-dimensional polymers were suggested by the authors

\* Bretteon and Sanders<sup>23</sup> note that the magnesium in bacteriochlorophyll *a* is five co-ordinate in acetone and six co-ordinate in methanol.



**Figure 3.** Proposed models for the chlorophyll *a* dimer: A, the Strouse model; B, the Shipman model; C, the skew model; D, the Fong model; E, the piggy back model; F, the back-to-back model

as possible models for the organization of antenna chlorophylls in plants, and in support of this hypothesis, calculations based on the exciton model of the spectral shift which occurs upon polymer formation were consistent with the spectrum of chlorophyll *in vivo*. In these polymers the C-9 C=O group of one molecule is situated under ring A of the neighbouring molecule as it is on the same face of the molecule as the C-10 substituent (Figure 3A). These one-dimensional polymers bear some relationship to the aggregate structure proposed from n.m.r. data for chlorophyll *b*,<sup>8</sup> in which rings A and C experience high-field shifts due to the neighbouring molecules. Thus it may be expected that this model would not reproduce the observed dimer (or aggregate) shifts for chlorophyll *a*. This is indeed the case and then calculated shifts based on the crystal geometry show no relationship with the observed solution shifts (Table 2).

No attempt was made to search for a better solution in view of the disparity between the observed and calculated shifts. However, this negative result is of some interest as it illustrates very clearly the profound differences in chlorophyll aggregation between the solid and solution states.

*The Shipman Model.*—Shipman *et al.*<sup>14</sup> proposed an alternative model for the structure of the special pair of chlorophyll *a* based on exciton theoretical considerations and on infrared and visible spectra of an ethanol adduct of chlorophyll in toluene, which shows the 700 nm absorption band of P700. In this structure, which is of  $C_2$  symmetry, the two equivalent chlorophyll *a* molecules are held together by two ROH ligands each of which is simultaneously co-ordinated to the magnesium atom of one chlorophyll molecule and hydrogen bonded to the

C-9 carbonyl of the chlorophyll molecule (Figure 3B). In this structure the inter-planar separation is given as *ca.* 3.6 Å. It can be seen immediately that rings C and E are above the same rings in the neighbouring molecule and therefore will experience a ring current shift in the dimer, but the C-10a ester methoxy group is *exo* to the dimer structure and would not be expected to show any appreciable effect. This is indeed seen in the calculated ring current shifts of this structure (Table 2); 10-H has also a very small ring current shift, being almost edge-on to the neighbouring molecule and in contrast the 5-Me group has a far greater calculated shift than observed. Again it was not felt necessary to perform a computational search of the dimer co-ordinates. Clearly the proposed structure would not account for the observed n.m.r. spectrum of chlorophyll *a* in chloroform, and there would appear to be no reason to suspect that the n.m.r. spectrum of chlorophyll *a* in toluene would be very different.\* The visible and infrared observations from which this model was in part deduced were from a moderately concentrated solution of chlorophyll in toluene ( $9 \times 10^{-2} M$  with the addition of 1.5 mol equiv. of ethanol).<sup>14</sup> The vapour phase osmometry studies<sup>11</sup> would appear to suggest that chlorophyll *a* at this concentration in toluene may well form larger aggregates than the dimer, even with a 1.5 mol excess of ethanol. (Note that >20 mol equiv. of methanol were required to dissociate the chlorophyll *a* complexes in the present study, which were in much more dilute solution.) Thus it may well be that the 700 nm absorbing species in these solutions is not a dimer but a much larger aggregate. This would certainly be in accord with similar recent observations of the bacteriochlorophylls *d*.<sup>24</sup>

*The Skew Dimer.*—A skew chlorophyll dimer was originally proposed on the basis of circular dichroism<sup>12</sup> and n.m.r.<sup>4</sup> studies in which the angle between the chlorophyll molecular planes was *ca.* 40°. This early suggestion has not been generally accepted, but more recently Kooyman and Schaafsma,<sup>15</sup> from measurements of nuclear relaxation times and chemical shifts, proposed a skew form of the chlorophyll dimer in which the two chlorophyll molecules are orthogonal with the C-9 C=O oxygen of one molecule co-ordinating directly to the magnesium atom of the other (Figure 3C). The theory of nuclear relaxation can only be applied to nuclei lacking any internal mobility; thus only the relaxation times of the three *meso* protons and 10-H were measured. As the general chlorophyll dimer has six internal degrees of freedom (the co-ordinates and orientation of one molecule with respect to the other), the determination of the dimer geometry from these measurements is under-defined, particularly as it is the differences between the nuclear relaxation times of the monomer and dimer which are utilized in these calculations. For this reason the authors also used ring-current chemical-shift calculations to further define the dimer geometry. They noted that only the general trend of the calculated ring current shifts agreed with their measurements, and noted also that as some of the protons in one of the chlorophyll molecules in the dimer are situated close to the ring plane of the other molecule, the dipole approximation may not be strictly valid at these distances.

On the basis of the figure shown, a model of the proposed dimer was constructed and the ring currents calculated. Our program can only calculate the ring current shifts of both molecules in the dimer in one operation if the molecular planes are parallel. Thus, for the skew model the shift of each molecule was calculated separately and the two data sets averaged. The calculated shifts (Table 2) do indeed follow the trends of the observed shifts for some of the protons measured, in particular

for the three *meso* protons, 1-Me, 3-Me, 5-Me, and 10-H. These were the only protons measured in ref. 15. However for most of the remaining protons measured here the calculated ring current shifts of the skew model are very different from the observed values. Note in particular the calculated shifts for the 10-OMe, which are positive (*i.e.* to low field), contrary to observation. This is indeed due to the closeness of approach of these protons to the neighbouring chlorophyll molecule in the dimer. These protons in one molecule are situated virtually in the ring plane of the other. This could give rise to unacceptably large steric repulsions between the two chlorophyll molecules; thus the model is not chemically acceptable.

The central problem of the skew dimer in which the C-9 C=O oxygen is directly co-ordinated, presumably along the lone-pair directions, with the adjacent magnesium atom, is a chemical one. The C-9 C=O is sterically shielded in the chlorophyll plane on one side by the C-10a ester group and on the other side by the C-5 Me. Thus, any attempt to co-ordinate the C=O oxygen directly with the magnesium atom of the adjoining molecule will result in considerable steric interactions between these neighbouring groups and the ring atoms of the adjoining molecule. In view of these factors, plus the poor agreement between observed and calculated shifts, we will not consider this structure any further.

*The Fong Model.*—This symmetrical head-to-tail structure was first proposed by Fong<sup>13</sup> to explain the original n.m.r. data. In this symmetrical dimer structure the C-10a C=O groups of both molecules in the dimer co-ordinate to the magnesium atoms of the adjacent molecule; thus, this is essentially a structure with  $C_2$  symmetry, both molecules in the dimer being in the same environment. (This is not a necessary condition for a face-to-face dimer, see later.) The central problem with this structure lies more in the steric requirements of the side chains than the n.m.r. data. Given that the C-10 ester group is in the *cis* orientation (*i.e.* the Me group eclipsing the C=O oxygen), which is the only conformation observed for all methyl esters, the distance of the methyl protons from the plane of the chlorophyll is *ca.* 2.5–3.0 Å, slightly more than that of the C=O oxygen (2.3 Å). Thus, in order for the C=O oxygen to approach close enough to be directly bound to the magnesium of the neighbouring molecule (*ca.* 2.6 Å above the plane of the neighbouring molecule), the protons of the ester methoxy group will experience quite unacceptable steric repulsions, with interatomic distances of *ca.* 2 Å with the neighbouring chlorophyll molecule.

Fong also proposed<sup>13</sup> a hydrogen-bonded structure analogous to this structure for the 'special pair' chlorophyll dimer with a bridging water molecule between each C-10 C=O and the magnesium atom of the neighbouring chlorophyll molecule (Figure 3D). Shipman *et al.*<sup>14</sup> estimated the separation between the ring planes in this structure as *ca.* 5.6 Å from molecular models. Thus, to test this proposed structure a computational search was carried out using the observed dimer shifts in Table 2. The restrictions imposed were that the chlorophyll ring planes are parallel at approximately the above inter-ring separation, and that the *x* axis is the two-fold symmetry axis of the dimer. This latter requirement is simply due to the co-ordinates of the C-10 C=O. On the co-ordinate system used here for chlorophyll *a* (1), the C=O oxygen of the methoxycarbonyl group lies on the *y* axis. The best solution obtained is given in Table 2 with the displacement co-ordinates, and it can be seen to reproduce the general trends of the observed shifts reasonably well. The inter-ring separation obtained of 5.0 Å is on the limit for this type of structure as the methyl protons of the 10-OMe group are only 2.2 Å above the plane of the neighbouring molecule, though there is some rotational mobility of the methoxycarbonyl group. Also, the agreement with the observed

\* Katz<sup>3</sup> notes that benzene and CCl<sub>4</sub> are very similar solvents in this regard.

data is not as good as could be expected, with the calculated shifts for some groups (*e.g.* 5-Me and 8-Me) in the wrong order.

A more cogent criticism of this model is that it does not explain many of the other observations on the chlorophyll *a* dimer. In particular the very similar complexation shifts for the pyrochlorophyll series, in which the critically important C-10 methoxycarbonyl group is absent, have no obvious explanation on the basis of this model, nor the strong infrared evidence associating the C-9 C=O with the dimer formation (see later).

*The 'Piggy-back' Model.*—This structure was originally proposed following an investigation with the ring current model using the earlier n.m.r. data.<sup>8</sup> It was apparent in this investigation that the presence of higher aggregates precluded a critical examination of this model; thus it is of some interest to examine the present data in this light. The analysis of the complexation data of Table 2 on the basis of this model was performed in a precisely analogous manner to that previously described. As before, we consider an unsymmetrical dimer structure in which the molecular planes are parallel to each other, in a head-to-tail configuration in which both molecules face the same way, *i.e.* the piggy-back structure (Figure 3E). The computation merely assumes this geometry and searches for the best agreement of the observed and calculated complexation shifts varying the displacement co-ordinates ( $x$ ,  $y$ ,  $z$ ) of one molecule with respect to the other, and also the angle of rotation ( $\theta$ ) of one molecule with respect to the other about the axis perpendicular to the molecular plane (the  $z$  axis). Given the above assumptions, these four parameters completely specify the basic dimer geometry, though of course the phytol side chain has a large number of possible conformations, and for this reason the complexation shifts of the phytol protons given in Table 2 are not used in the scanning procedure. However, using the other twelve dimer shifts given in Table 2, the scan converged to a single solution with the displacement co-ordinates and calculated shifts given in Table 2. The r.m.s. error of the observed and calculated shifts is 0.08 p.p.m. and the largest single deviation is 0.21 p.p.m. over a range of measured values from 0 to 1.3 p.p.m. Thus the agreement is good, given the basic assumptions of the model. Owing to the intrinsic properties of the ring-current field, which changes only very gradually with distance over the face of the molecule, falling off more rapidly at the edges of the molecule and also with respect to the  $z$  axis, the definition of the dimer geometry obtained is not as precise as may have been anticipated. The uncertainties in the displacement co-ordinates are *ca.*  $\pm 0.5$  Å with an equivalent value for  $\theta$  ( $5^\circ$ ).

The values of the dimer co-ordinates are identical with the values previously obtained, though the former were much less accurate *e.g.* the inter-planar separation was quoted as 5.0–7.0 Å. The more accurate geometry obtained here does allow some specific deductions to be made. Inspection of molecular models shows that the C-10 C=O points directly towards the central magnesium of the adjoining molecule. (We assume a *trans* coplanar conformation of this C=O and 10-H, as found in the crystal.) However, the inter-planar separation of 6 Å is far too large to allow direct co-ordination of this group with the magnesium (assuming that the magnesium lies 0.4 Å out of the molecular plane towards the second chlorophyll molecule gives a Mg...O=C distance of 4.0 Å). The geometry found would appear to be optimum for a co-ordinating water molecule bound to the magnesium to hydrogen bond to the carbonyl oxygen of the C-10 group. On this model the C-9 C=O, though situated over the central magnesium atom, would appear to be too distant to be directly involved in the dimer bonding. It would require a hydrogen-bonded chain of at least two water molecules to attach this group to the adjoining magnesium atom. This raises the central problem of this dimer structure, as

with the Fong structure, which is to explain the very similar results obtained with the pyrochlorophylls, in which the C-10 methoxycarbonyl group is absent.

*The General Face-to-face Model.*—The above examination of the various proposed models of the chlorophyll dimer shows that there is no completely satisfactory model of the dimer geometry which will both quantitatively reproduce the observed dimer shifts and also provide a reasonable explanation of the results of other chemical observations. Thus it was felt that a general computational search of other possible structures may be appropriate.

The Fong model of the face-to-face dimer possesses  $C_2$  symmetry with both molecules of the dimer in identical environments. However, the general face-to-face dimer does not possess any symmetry and the two molecules may be in very different environments. Recently the necessary geometric operations for performing the calculation of the ring-current shifts of both molecules in such a dimer were described.<sup>25</sup> In these calculations, it is necessary to invert one of the chlorophyll molecules with respect to the other, and it is this inversion operation which differentiates the various face-to-face models from the piggy back model, which is a back-to-face structure. However the computations still retain the assumption of two parallel molecular planes in the dimer, reducing the number of internal degrees of freedom from six to four (one rotation and three displacement co-ordinates).

There are of course two possible ways of combining the asymmetric chlorophyll molecules in a face-to-face structure, depending on which face of the molecule is inside the dimer (*i.e.* *endo*) and which is on the outside. It is convenient to describe these as the face-to-face model, in which the C-10 methoxycarbonyl groups are *endo* to the structure, as in the Fong model, and the back-to-back model in which both the methoxycarbonyl groups are *exo*. Both of these calculations were performed and the four parameters defining the molecular structure were varied until the best agreement of the calculated and observed shifts was obtained.

The two calculations gave completely different answers as follows. In the face-to-face model, the computational search started with the Fong structure, using all the observed dimer shifts of Table 2, except those of the phytol side chain, exactly the same procedure as for the piggy-back model. The best agreement however was found for the Fong symmetrical structure, although this symmetry condition was not imposed during the calculations. Starting the calculations from other structures produced the same result, establishing that this was not just a local minimum in the parametrization. This structure has already been considered.

For the other possible structure, the back-to-back model, a completely different result emerged. A chemically reasonable starting structure was input with both C-9 C=O positioned near to the magnesium atom of the adjoining molecule. This involves a displacement of the origin plus a rotation of the molecule about the  $z$  axis of *ca.*  $45^\circ$ . This structure however did not give any agreement between the observed and calculated shifts, but the computational search converged smoothly to a quite different structure, with very reasonable agreement between the observed and calculated shifts (Table 2). The r.m.s. error for the twelve measurements involved in the iteration was 0.08 p.p.m. with the largest deviation of 0.14 p.p.m. for the C-10 methoxycarbonyl group, which may very probably not be in the single conformation found in the crystal.

The structure obtained does not possess  $C_2$  symmetry and the two molecules are in very different environments. In this structure the inter-planar separation is *ca.* 4.8 Å, but the two molecules are not rotated with respect to each other, simply inverted and displaced (Figure 3F). The C-9 C=O group of one

molecule is indeed positioned in the neighbourhood of the magnesium atom of its partner, but the inter-ring separation is such that this would need to be *via* an intervening water molecule (the C=O to Mg distance is *ca.* 4.9 Å). The other C-9 C=O group in this structure is far removed from the neighbouring magnesium atom and plays no part in the binding. Furthermore both the C-10 methoxycarbonyl groups, being *exo* to the dimer structure, also play no part in the binding. In the structure found, the carbonyl group of the C-7 propionate side chain of the second molecule can approach close to the magnesium atom of its partner, thus contributing to the binding energy of the dimer (calculations give a separation of *ca.* 4 Å, but this is very dependent on the precise conformation of both ring D and the propionate side chain).

There is strong support for the involvement of the C-7 C=O in the binding of the chlorophyll dimer from the observed complexation shifts of the phytol protons (Table 2). Those protons near the C=O group show significant upfield shifts, though these shifts rapidly decrease with increasing distance of the protons from the ester group. Furthermore, the methyl protons of the C-7 methoxycarbonyl group in methyl chlorophyllide *a* also show considerable complexation shifts (*ca.* 0.85 p.p.m.).<sup>3</sup> These shifts can be qualitatively reproduced by the above conformation of the propionate side chain in which the C=O group is near to the neighbouring magnesium atom.

The proposed back-to-back structure, as it does not involve any co-ordination of the C-10 methoxycarbonyl groups with the magnesium, does provide a reasonable explanation for the similar complexation shifts observed in the pyrochlorophyll series in which the C-10 methoxycarbonyl group is absent, and also for the results of infrared investigations which are strong evidence for the involvement of the C-9 keto group in the dimer formation. In non-polar media the intensity of the free C=O band of chlorophyll *a* at 1 695 cm<sup>-1</sup> attributed to the C-9 C=O is diminished by half and a new band at 1 652 cm<sup>-1</sup> appears. The results find a reasonable explanation in terms of two kinds of C=O group in the dimer, one free and one co-ordinated to the magnesium, exactly as found in the back-to-back dimer.

## Conclusions

Of the various chlorophyll *a* dimer structures previously proposed, only the Fong symmetric dimer and the 'piggy back' model give any reasonable agreement with the complexation shifts presented here. The Fong model gives moderate agreement (r.m.s. difference, observed *vs.* calculated shifts, 0.13 p.p.m.) and the computationally best solution gives somewhat too-close interatomic distances to be chemically acceptable. The piggy back structure gives significantly better agreement (r.m.s. difference, 0.09 p.p.m.) and also a chemically reasonable structure with no unacceptably close interatomic distances. Both the Fong and the piggy back structures fail to account for the similar complexation shifts observed in the pyrochlorophyll series, and for evidence implicating the C-9 C=O in the dimer interaction.

The proposed back-to-back model gives marginally better agreement with the observed shifts than any other model (r.m.s. 0.08 p.p.m.), is a chemically reasonable structure in that there are no close interatomic distances, and also does account very straightforwardly for the pyrochlorophyll and infrared enigmas. What is however not immediately obvious is why this structure should be preferred, if indeed this is the chlorophyll dimer structure, over the other proposed structures. It may be that this structure provides the only means by which the C-9 C=O can approach close enough to co-ordinate, *via* a water molecule, to the magnesium atom of the adjoining molecule. Any similar approach from the other face of the molecule immediately leads to steric repulsions with the C-10 methoxycarbonyl group. This

group is absent in the pyrochlorophyll system in which both faces then become equally accessible, and it would be of some interest to pursue a similar in depth study in this series. The studies are in progress.

The back-to-back structure does result in a closer dimer structure than the other models, the inter-ring separation is *ca.* 4.8 Å ( $\pm 0.5$  Å), and this would enhance the attraction between the chlorophyll molecules themselves. The stacking tendency of the chlorophyll ring is clearly evidenced by the similarity in the crystal structures of methyl pheophorbide *a* and methyl chlorophyllide, even though there is no magnesium atom in the former.

It is of interest to note the relationship between the proposed back-to-back dimer (Figure 3F) and the crystal structure model (Figure 3A). There is virtually the same orientation of one chlorophyll molecule with respect to its partner in both structures. The difference in the structures is due to the different orientation of the second molecule. In the crystal this molecule is merely translated from the first, thus providing a continuous repeating unit. In the back-to-back model, on the contrary, the second molecule is inverted, resulting in a much more condensed structure. This does suggest that the primary co-ordination of one C-9 C=O with the adjoining magnesium atom *via* an interstitial water molecule is the dominant complexation mechanism in chlorophyll *a*.

It would be of interest to know whether the back-to-back model would account for any of the results obtained from the other physical techniques used to investigate chlorophyll aggregation. However, this is beyond the scope of the present study. It should finally be noted that the back-to-back structure cannot form similarly constituted aggregates. However, with one free C-9 C=O group and an adjoining C-10 methoxycarbonyl group, aggregates with the piggy back structure could easily 'grow' from this asymmetric seed. The relevance of this system to *in vivo* chlorophyll may be questioned, but the combination of a back-to-back dimer with a piggy back type of aggregation does constitute a chemically acceptable model for chlorophyll antennae.

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